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# STUDIES OF ANTHOCYANIN SYNTHESIS IN LEAVES OF *PRUNUS VIRGINIANA* L. *MELANOCARPA* (A. NELS.) SARGE. 'SCHUBERT' AND *EUONYMUS ALATUS* SIEB. *COMPACTUS*

*by*

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ABSTRACT

Severe root pruning in the spring, girdling, and the application of anti-gibberellin-like compounds greatly increased anthocyanin pigmentation of Schubert chokecherry, *Prunus virginiana* L. *melanocarpa* (A. Nels.) Sarge. 'Schubert', leaves. However, greenhouse experiments involving a manipulation of temperature, photoperiod, and soil moisture levels failed to stimulate the synthesis of anthocyanin in leaves of the dwarf-winged burning bush, *Euonymus alatus* Sieb. *compactus*.

Leaves of the dwarf-winged burning bush were shown to be dependent upon light of short wavelengths for anthocyanin synthesis. Therefore, the filtering effect of glass on this portion of the spectrum, probably accounted for the lack of results in the greenhouse experiments. Interestingly though, leaves on rooted terminal cuttings of this plant were found to synthesize more anthocyanin than did basal cuttings from the same shoot.

The content of anthocyanin, chlorophyll, and leucoanthocyanin in leaves of the Schubert chokecherry is dependent upon leaf maturity. Leaves on shoots showed a progression of increased content of these compounds corresponding to the maturity of the leaves. It was also observed that at 16 1/2 weeks after the onset of bud expansion, the fourth basal leaves on shoots contained

64.6 µg anthocyanin per $cm^2$. At this time, the chlorophyll content had decreased slightly below the 62.8 µg per $cm^2$ maximum observed six weeks earlier. As the anthocyanin pigmentation increased in the leaves, a significant decrease in net assimilation of $CO_2$ was observed.

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## INTRODUCTION

Plants displaying anthocyanin pigmented leaves are often of special ornamental value. The timing of this pigmentation varies according to the particular plant species and the prevailing environmental conditions. The Schubert chokecherry, *Prunus virginiana* L. *melanocarpa* (A. Nels.) Sarg. 'Schubert' and the dwarf-winged burning bush, *Euonymus alatus* Sieb. *compactus*, are prime examples of plants showing variation.

The Schubert chokecherry is a selection of the western chokecherry, a native of many areas in the prairie provinces. It is a small deciduous tree that is known for its development of deep purplish-green leaves during the growing season. The anthocyanin pigmentation starts soon after the leaves have fully expanded and developed a deep green color. Consequently, during the early part of the growing season, the oldest to youngest leaves on the same branch may show a progression from purplish-green to very light green.

The dwarf-winged burning bush, on the other hand, may develop brilliant scarlet red leaves in the autumn. This intense anthocyanin pigmentation display follows chlorophyll degradation. It is evident in Southern Ontario and in many areas of the United States. However, in the

Edmonton area, as in the rest of Alberta, this shrub is not known to produce such a brilliant display of autumn coloration. Anthocyanin pigmentation does occur following light frosts. However, the leaves abscise before full coloration is reached. Consequently, in the Edmonton area, the dwarf-winged burning bush is not held in as high esteem as an ornamental as it is when grown under environmental conditions more suitable for full autumn coloration.

Blank (1958) noted that leaves containing anthocyanins contained more chlorophyll than did green leaves. It has not been reported if the chlorophyll content of Schubert chokecherry leaves changes in conjunction with the synthesis of anthocyanins. The effect of anthocyanins on the photosynthetic and respiratory rates of Schubert chokecherry leaves has also not been determined.

Cultural practices are known to affect the synthesis of anthocyanins in many plants (Weeks *et al*, 1958, Toy, 1969). However, to my knowledge, nothing has been done to determine the effects of cultural practices on the synthesis of anthocyanins in the leaves of Schubert chokecherry. The same is believed true for the dwarf-winged burning bush.

Lee and Tukey (1971) determined that late summer rains could delay and reduce autumn coloration of the dwarf-winged burning bush in New York. This obviously is not the reason for the greatly reduced autumn coloration by this plant in the Edmonton area.

There are many unanswered questions regarding anthocyanin pigmentation of leaves of the Schubert chokecherry and the dwarf-winged burning bush. This study, therefore, was undertaken with the following objectives in mind: (1) to study some factors affecting the anthocyanin pigmentation of their leaves, and (2) to investigate factors associated with this pigmentation.

## LITERATURE REVIEW

Anthocyanin pigmentation of leaves may occur at several stages of leaf maturity. Its occurrence, however, is not always apparent. The visual aspect of this pigmentation may be affected by co-pigmentation or it may be masked completely by chlorophyll. In such cases, it is not noticeable until subsequent chlorophyll degradation. This is especially true in the case of autumn coloration of leaves.

Harborne (1965) classifies anthocyanin pigmentation into three categories: transient, permanent, and autumnal. These categories will be considered separately.

During the spring, many higher plants develop anthocyanin in the young leaves. This is known as transient pigmentation. It usually disappears rapidly with leaf expansion. Price and Sturgess (1938) list approximately 200 species in 110 genera that exhibit transient pigmentation.

Transient pigmentation may also be the result of an accumulation of excess sugars, disease infection, or environmental or cultural stress. High intensity light, nutrient deficiency, or low temperature are examples of the stress factor. Transient pigmentation may also be brought on artificially by wounding or

treatment with growth regulators (e.g. Harborne, 1965, Blank, 1958).

Permanent leaf coloration other than green is rather uncommon in nature. Harborne (1965) regards most plants exhibiting this phenomenon as variants of the common green forms. In addition, he concludes that the pigment lacks function. In contrast, Daubenmire (1959) states that anthocyanin pigmentation acts as a reflective screen which protects underlying tissues from red radiation. Similarily, Caldwell (1968) regards anthocyanin pigmentation in alpine plants as a protective measure to prevent tissue damage as a result of the high ultraviolet radiation.

Autumnal coloration is generally associated with the accumulation of sugars resulting from the degradation of starch during senescence. A correlation often exists between levels of carbohydrates and anthocyanin synthesis. Street and Cockburn (1972) conclude that this is not a causitive relationship but rather the result of a greater availability of substrate material.

## Physiological Factors Affecting the Biogenesis of Anthocyanins

The synthesis of anthocyanins by higher plants is dependent upon several environmental factors. These factors have been reviewed by Blank (1958), Bogorad (1958), Kandeler (1960), Neish (1960), Siegleman (1964), and Walter (1967).

A. Light

It has long been known that light is one of the most important environmental factors affecting synthesis of anthocyanins. For example, man has long recognized that the reddest apples are always found on the sunniest side of the tree. Light is involved in photomorphogenetic mechanisms as well as photochemical reactions. Photoperiod, light quality, and light intensity are all known to affect the synthesis of anthocyanins.

Autumn coloration and leaf senescence are photoperiodic responses of some plants. According to Hemphill and Tukey (1973), photoperiod and light intensity both influence autumn coloration of *Euonymus alatus compactus*. They report that anthocyanins are not synthesized following an inductive photoperiod if the plants are either exposed to low light intensity or not exposed to low temperatures.

Similarly, light quality is responsible for many plant responses. Photochemical reactions requiring energy from different parts of the light spectrum have been reviewed by Withrow (1959) and summarized by Blackwell (1966). Phototropism occurs at 300 to 500 mμ with maxima in the ultraviolet at 379 mμ and in the blue at both 445 and 475 mμ. Photosynthesis and the synthesis of chlorophyll both occur at 400 to 500 mμ and 600 to 700 mμ. However, photosynthesis is activated chiefly by blue light near 435 mμ and red light near 650 mμ. These two photochemical reactions are relatively inactive in green

light near 560 mμ.

Voskresenskaya (1972), reviewing the role of blue light in carbon metabolism, notes that blue light results in a reduction of carbohydrates, especially starch, through increased glycolysis. In contrast, the synthesis of amino acids, organic acids, and ribonucleic acids is increased by blue light. Blue light is also associated with enhanced biosynthesis of protein and, as noted earlier, chlorophyll, particularly chlorophyll b.

Research by Thomas *et al* (1970) indicates that plant material grown with a long exposure to blue and green light of low intensity produces more carbohydrates than those grown in red light. However, short exposures to red light of high intensity results in the highest production of carbohydrates.

The effects of light quality on the synthesis of anthocyanins vary with the plant species. These effects and variations have been studied and reviewed by several authors: Arthur (1936); Grisebach (1965); Leopold (1964); Mohr (1962, 1969); vander Veen and Meijer (1959); and Walter (1967).

The phytochrome system and high energy reaction (HER) are shown by Mohr (1969) to be important in the synthesis of anthocyanins in mustard seedlings. Arthur (1936) and vander Veen and Meijer (1959) conclude that strong blue light is important for the coloration of apple fruit. In contrast, Siegelman and Hendricks (1957) report that red radiation is responsible for the formation of

anthocyanins in red cabbage. The action spectra for the synthesis of anthocyanins in these three species is compared by Siegelman (1964) in his review of techniques of action spectroscopy. The various maxima are shown to be clustered in the blue and red regions of the spectrum.

The biosynthesis of anthocyanins follows the shikimic acid pathway. Phenylalanine ammonia-lyase (PAL) is a major enzyme component of this pathway (Grisebach 1965). The synthesis of PAL is controlled by the phytochrome system in some plants. However, other plants show no dependence on light for the synthesis of this enzyme, and still other plants possess a blue light high energy reaction (HER) system for the synthesis of PAL (Zucker 1972).

Hadwiger and Schwochau (1971) report that short-wave ultraviolet light (254 mμ) stimulates synthesis of PAL in pea tissue, whereas long-wave ultraviolet (366 mμ) and blue light do not. In contrast, Creasy (1968) notes that blue light stimulates PAL synthesis in strawberry leaf discs more so than does light of other wavelengths.

The intensity of light reaching plant leaves varies at different altitudes and latitudes and from season to season (Moon, 1940, Robertson, 1966, Brooks and Miller, 1963). The difference in light intensity is greater at some wavelengths than at others and does affect the synthesis of anthocyanins (Caldwell 1968).

There are three means by which a parallel beam of monochromatic radiation is depleted while passing through the atmosphere (Collingbourne 1966). The first is by what is known as Rayleigh scattering and is responsible for the redness of sunset and the blue of the sky. Light waves are scattered by air molecules and other particles which are relatively smaller than their wavelengths. The scattering is proportional to the density of the scattering medium and is inversely proportional to the fourth power of the wavelength. Thus, short wavelengths are affected most.

Secondly, atmospheric gases absorb radiation. Radiation between 200 mμ and 400 mμ is absorbed by ozone, oxygen, and water vapor. Short wavelengths (less than 330 mμ) are absorbed primarily by ozone. As a result of this, the shortest wavelength detected in solar radiation at the earth's surface is 290 mμ.

Atmospheric particles equal to or greater than the wavelength of the radiation both absorb and scatter radiation. This is the third means of depletion of radiation passing through the atmosphere. Clouds, dust, and atmospheric pollution products are all responsible.

The depletion of radiation by all three methods depend upon the amount of air mass through which the light passes. Seasonal fluxes are a direct result of the variation in the declination of the sun, a consequence of the tilt of the earth's axis. Obviously, the daily

total solar radiation at a given latitude outside the tropics is maximum at the summer solstice.

Data on the effect of altitude, latitude, and season on the intensity of light of various wavelengths reaching the earth's surface is, unfortunately, sparce. Measurements reported by Robertson (1966) indicate that there are seasonal and latitudinal variations in light intensity of the shorter wavelengths, that is, ultraviolet and blue. This is a result of the changing solar elevations.

The change in ultraviolet radiation with altitude was investigated by Caldwell (1968). His measurements show that the intensity of ultraviolet radiation is up to 50% higher at 4350 m elevation than at 1670 m. Caldwell notes that earlier researchers detected an even greater increase in ultraviolet radiation with increasing altitude. Caldwell's results are also consistent with those reported by Brooks and Miller (1963). They report that the intensity of short wavelengths decreases with an increase in air mass.

Plants grown in the artificial environment of a glass greenhouse also receive a different light spectrum than do those grown under natural conditions. Glass used in greenhouses is known to reduce the intensity of short wavelengths. This is especially true for ultraviolet radiation (Popp and Brown, 1936). Short wavelengths are further reduced by a decrease in the angle of incidence of the sun to the glass (Dietz, 1963) which occurs during

evenings and late in the growing season. It is reduced to 80% in June but as low as 40% in December (Scholte-Ubing, 1961).

Shantz, cited by Popp and Brown (1936), notes that greenhouse-grown plants have less anthocyanin pigmentation than field-grown plants. This is consistent with Popp and Brown's report that ultraviolet, violet, and blue light are important in the synthesis of anthocyanins. They, too, note the filtering effect of short wavelengths by glass.

B. Temperature

Exposure to low temperature induces anthocyanin synthesis in leaves and fruits of some species of plants. Furthermore, low temperatures usually induce leaf senescence and abscission (Abbot, 1970, Tromp, 1970).

Lee and Tukey (1971) observed that exposure to a constant temperature of 5°C greatly increased anthocyanin synthesis in *Euonymus alatus* plants previously grown in a warm greenhouse or controlled environment chamber.

Cool nights in late summer have long been associated with enhanced coloration of apples. Research reviewed by Walter (1967) indicates that cool nights (50°F) associated with warm days (77°F) was more beneficial than cool nights with cool days (55°F). Hot days (89°F) inhibited anthocyanin synthesis. This is consistent with results published by Marten, *et al* (1970). They found that synthesis of anthocyanin in *Begonia gracilis* V. "*Garmen*" is greatly reduced at high temperatures. However, Walter

(1967) noted that low average absolute humidity in July had a greater effect on synthesis of anthocyanins in apples than did the average temperature. Chandler (1958) noted that anthocyanin development in blood oranges is reduced when they are grown in warm and humid areas.

C. Soil Moisture and Root Growth

Root growth and the availability of soil moisture may influence the growth of leaves as well as affect their metabolism (Kongsrud, 1969, Stanhill, 1957). For example, the movement of water, mineral nutrients and growth hormones, such as cytokinins and gibberellins may be altered by these factors (Cleland, 1969, Wareing, 1970). Roots are known to synthesize gibberellins and cytokinins and both of these hormones are involved with senescence (e.g. Lang, 1970; Wareing, 1970; Crozier and Reid, 1971).

Blank (1958) points out that drought may enhance coloration of leaves of some tropical trees but with some other plants anthocyanin synthesis may be reduced by drought. Toy (1969) observed that irrigation of *Acer truncatum* resulted in an increased but delayed autumn coloration. Toy also found that senescence was delayed by summer irrigations.

The Effects of Ringing

Phloem disruption by girdling or ringing is a technique that can be used to control growth and to induce early fruiting and more intense fruit coloration. The use of this practice impedes the basipetal translocation

of the synthesized organic materials. Carbohydrates, amino acids, auxins, and other growth regulators are known to accumulate above the disruption (Kozlowski, 1971b; Stoltz and Hess, 1966; Wilson, 1970). According to Kozlowski, the organic solutes tend to diffuse into the xylem and are then retranslocated acropetally.

Skene (1972) reports that ringing reduces the cytokinin level of grape shoots. Weaver and Poll (1965) note an increase in some gibberellins while others are depressed by ringing. They postulate that, following ringing, there may be a transformation between the gibberellins. Skene (1972) concludes that stress conditions that follow ringing may result in the inactivation of cytokinins.

Ringing may also interfere with the movement of carbohydrates as noted earlier, and this may influence anthocyanin synthesis. However, the many effects attributed to carbohydrate variation following ringing could also be due to differences in the levels of growth substances (Luckwill, 1970).

Effect of Anthocyanins on Leaf Net Assimilation Efficiency

Anthocyanins may have an effect on the role or efficiency of other pigments. The rate of photosynthesis and respiration may be altered by the presence of anthocyanins. Anthocyanin pigmentation, in turn, is often masked by other leaf pigments, especially chlorophyll.

Maximum photosynthetic capacity is usually

attained at the time of full leaf expansion. The subsequent photosynthetic rate tends to decline until leaf abscission (Kozlowski 1971a). Respiration, in contrast, is highest in young leaves and gradually decreases with age. Interestingly, however, it may suddenly increase at the time of senescence. (Kozlowski, 1971a).

Blank (1958), in his review of research on the relationship of anthocyanins to photosynthesis, observes that the chlorophyll content of leaves containing anthocyanins may be lower than that of green forms. However, Blank notes that in some pigmented leaves studied, the chlorophyll content is greater. However, the rate of photosynthesis may be decreased as a result of the light filtering effect of anthocyanins in the epidermal cells. This author concludes that the difference between rate of assimilation in green leaves versus red leaves is often the result of the difference in chlorophyll content of the two leaf forms.

Anthocyanin pigmentation is known to have an effect on respiration rates of tissue. Shulak and Hapitan (1969) report a higher respiration rate under red apple epidermis than under green epidermis. Similarly, Bjorkman and Holmgren (1958) found that alpine and subalpine ecotypes have increased respiration rates and this is associated with increased anthocyanin synthesis. Conversely, lowland ecotypes have lower respiration rates and lower anthocyanin synthesis.

Kelskhoveli and Japaridze (1972), in their study of respiration intensity of red and green forms of tree leaves, observed that respiration was 1.5 to 2 times higher in red leaves than in corresponding green leaves. In addition, respiration decreased during vegetative growth. These authors suggest that anthocyanin pigments may have a role in the oxidation reduction processes.

Physiological Effects of Gibberellins

The most typical response of higher plants to gibberellins is enhanced stem elongation (e.g. Marth *et al*, 1956; McVey and Wittwer, 1958). Shoot growth is clearly controlled by the interaction of the major plant hormones: gibberellins, auxins, cytokinins, and dormins (Luckwill, 1970, Possingham, 1970).

Leaf senescence may be the result of a change in enzymatic activity controlled by hormones or be the result of hormone deficiency (Kozlowski, 1971a).

Brian *et al*,(1956) observed that autumn coloration and leaf fall of some deciduous trees is delayed by gibberellic acid sprays.

Growth retardants are very useful in the study of gibberellin physiology. Lang (1970) reviews their use in the regulation of gibberellin biosynthesis. The growth retardants B-9 (N-diemethylamino succinamic acid) and Phosphon-D (tributyl-2-4-dichlorobenzylphosphonium chloride) are believed to be regulators of the biosynthesis or action of gibberellins in some plants (Pharis, *et al*,

1970; West and Fall, 1970).

Anthocyanin Synthesis and Leucoanthocyanin Content

Research on the role of leucoanthocyanins in the biogensis of anthocyanins has resulted in opposing views. Bogorad (1958) concludes from experimental observation that leucoanthocyanins are possible intermediates in the biogensis of anthocyanins. Robinson (1967) is of the opinion that in some plants anthocyanins are synthesized from leucoanthocyanins, whereas in others they are both end products of parallel pathways. However, others (e.g. Harborne, 1964) believe that leucoanthocyanins are involved in the biosynthesis of tannins because of their common occurrence in woody species.

Regardless of the biosynthetic role of leucoanthocyanins, they are clearly associated with the degree of maturity of leaves (Hillis, 1956). Research by Hillis and Swain (1959) reveals that the leucoanthocyanin content in leaves of *Prunus domestica* increases rapidly and then gradually decreases throughout the growing season. Similarly, Lee and Tukey (1971) determined that leucoanthocyanin in mature leaves of *Euonymus alatus compactus* gradually decreases during a six-week period in the late summer. However, they noted a sharp increase in leucoanthocyanin content during the following two week period. At the end of the eight week period, these researchers could induce rapid anthocyanin synthesis by exposing the plants to low temperatures (5°C).

# MATERIALS AND METHODS

## I. Plant Material

### A. Schubert Chokecherry

Mature trees and potted three year old seedlings comprised the plant material for research associated with the Schubert chokecherry (*Prunus virginiana* L. *melanocarpa* (A. Nels.) Sarg. 'Schubert'.

The mature trees were approximately 4 m in height and consisted of trees growing on the University of Alberta Campus as well as trees growing in the University of Alberta nursery. Trees of the latter group were either transplanted to the University of Alberta campus during the fall of 1972 or transplanted to the University of Alberta Research Centre during the early spring of 1973. Transplanted trees received severe root pruning but little or no top pruning and are referred to in the text as those receiving severe root pruning the previous fall and those receiving severe root pruning in the spring. The control trees were those left growing in the nursery.

Seedlings were dug from the University of Alberta nursery in the fall of 1972 and potted in 20 cm plastic pots using a mixture of sand: peat, 1:1, v/v. These saplings had approximately one-third of their tops pruned off at the time of potting. One month later, the plants

were stored at 5°C until required. These plants are referred to as potted plants in the text.

B. Dwarf-winged burning bush

Experiments with the dwarf-winged burning bush (*Euonymus alatus* Sieb. *compactus*) involved uniform 45 cm plants obtained from nursery stock in Ontario on May 3, 1972, and one-year-old plants obtained from rooted cuttings of these plants. The former plants were transplanted into raised greenhouse benches divided by cross-boards into 60 x 90 cm plots. The soil depth was 30 cm. The latter plants were grown in 10 cm clay pots. Additional rooted cuttings were obtained from plants on the University of Alberta Campus. All plants were grown in a mixture of soil: peat: sand, 2:1:2, v/v/v with a pH of 6.5 unless stated otherwise.

II. Experimental Procedures

Unless specified otherwise, the methods described herein applied to all experiments. Colorimetric measurements were made using a Bausch and Lomb Spectronic 20 Spectrophotometer.

A. Extraction procedures

1. Anthocyanin

Ten leaf discs were steeped in 10 ml of 1% HCL in absolute methanol at 5°C for 24 hours. The anthocyanin content of the extract was measured using the method of Swain and Hillis (1959) and expressed as micrograms cyanin per $cm^2$ of leaf area.

2. Leucoanthocyanin

Each sample was boiled in 10 ml of methanol for 20 minutes. The marc was ground in a mortar and re-extracted five times with 10 ml of hot methanol for 20 minutes each. The supernatants after centrifuging were combined and taken to dryness on a rotary evaporator under vacuum at 40°C. The residues were redissolved in 10 ml of distilled water and the chlorophyll was removed by partitioning with 3 ml of petroleum ether. The leucoanthocyanin content was measured using the method of Swain and Hillis (1959) and expressed as optical density at 550 mμ using 1 ml of extract per sample.

3. Chlorophyll

Chlorophyll was extracted from each sample by grinding in a mortar with 2 ml of 80% acetone and filtering. The chlorophyll in the filtrate was partitioned into 2 ml of petroleum ether and decanted. This was repeated and the combined petroleum ether solutions taken to dryness *in vacuo* at 40°C. The residue was redissolved in a known volume of 80% acetone. Chlorophyll was estimated spectrophotometrically using the coefficients of Vernon (1960) and expressed as total chlorophyll per $cm^2$ of leaf area.

B. Experiments involving the Schubert chokecherry

Schubert chokecherry leaves were studied to determine if the pigment content of the leaves was dependent upon their location on the shoot and if the

chlorophyll content changed as the anthocyanin content of the leaves increased. The results of this experiment led to a study of the effect of anthocyanin pigmentation on leaf net assimilation efficiency. Additional experiments were conducted to determine the changes in pigment concentration over a 12-week period following the start of anthocyanin synthesis, as well as the effects of phloem disruption, severe root pruning, and anti-gibberellin-like growth regulators on anthocyanin, chlorophyll and leucoanthocyanin accumulation in the leaves.

1. Sampling and sample preparation

Sampling consisted of harvesting five leaves from each of five trees. Each leaf was the fourth basal leaf on terminal shoots of branches. Leaves were collected between 8 and 9 a.m. and kept in plastic containers at 5°C prior to analysis being done the same day.

The combined sample was made up of discs cut in pairs from each of the five leaves per tree. Discs were cut from the laminae of leaves by means of a 1.0 cm diameter cork borer.

2. Relative leaf position on shoot

Uniform terminal shoots were harvested at the time when basal leaves appeared fully pigmented and terminal leaves appeared to lack anthocyanins. Ten shoots were divided into 2 groups of 5 each. Leaves from 5 locations on each group of branches were sequentially numbered from 1-5 and were analyzed for anthocyanin, leucoanthocyanin, and

chlorophyll contents. Leaves from the 5 basal to terminal segments are referred to respectively as leaves from segments 1 to 5 in the text.

3. Leaf net assimilation efficiency

Respiration and photosynthetic rates of one leaf showing no anthocyanin pigmentation and of two leaves partially anthocyanin pigmented were measured with a UNOR (Marhak) infra-red gas analyzer (IRGA) using the method of Sestak *et al* (1971). The IRGA was used as a differential analyzer spanned 30 ppm using standard gases. The $CO_2$ content of the standard gases was determined previously using gases mixed with a Wostoff pump train as described by Bate *et al* (1969).

An open gas-exchange system was used. The incoming air stream was divided such that one portion passed through the enclosure containing the plant material and the other passed directly to the IRGA. Enclosures were rectangular plexiglass cuvettes 20.5 x 12.5 x 2 cm. The air stream passed diagonally through the cuvette and was varied so that the change in $CO_2$ concentration was not greater than 15 ppm. This required air flows of 4 to 7 liters per minute.

Leaf and air temperatures were measured using copper-constantan thermocouples (.003 mil). Leaf thermocouples were mounted on hair clips, which were used to hold them in place. Temperatures were recorded continuously. The ambient air temperature was 23.5°C. The

illuminated leaf temperature was 26.5° ± 0.5°C.

Leaves on cut shoots supported in water were mounted in horizontal cuvettes. A bank of six - 75 watt Westinghouse Reflector-type Flood Lamps was placed 20 cm above the leaves. Heat radiation was filtered out by 3 cm of water. Light intensity reaching the leaves was $3.4 \times 10^5$ ergs/cm$^2$/sec as measured by a YSI Kettering Model 65 Radiometer. Dark respiration was measured by covering the cuvettes with aluminum foil.

4. Pigment development

Sampling of leaves commenced when the three basal leaves of shoots showed red pigmentation but the fourth lacked such pigmentation. This date, June 19, 1973, corresponded to approximately 5 1/2 weeks after the onset of bud expansion. Sampling continued weekly for six weeks. A final sample was taken at the end of 12 weeks. The concentrations of anthocyanin, total chlorophyll and leucoanthocyanin in the sampled leaves were determined for each sampling period.

5. Effects of phloem disruption

On May 12, 1973, the time of bud expansion, a ring of bark about 5 mm wide was removed about 2 cm below the terminal bud on several branches on each of four established trees. Care was taken to avoid damage to the woody layers. A commercial wound dressing was applied to the ringed area.

Samples were harvested 5 1/2, 6 1/2 and 10 1/2

weeks after the treatment commenced and analyzed for anthocyanin, leucoanthocyanin, and chlorophyll contents.

6. Effects of root pruning

The following treatments were given to 3 groups of 4 trees: (1) established trees (control), (2) similar trees transplanted the previous fall, and (3) similar trees transplanted early in the spring. Transplanted trees received a minimum of top pruning and severe root pruning.

Immediately following sampling on June 26, 1973, imprints of each leaf were made using a Xerox 1000 photocopying machine. Leaf area of these imprints were measured using a planimeter. The average of 2 measurements per leaf was used. Anthocyanin content of the samples was determined.

7. Effects of anti-gibberellin-like growth regulators

Potted Schubert chokecherry saplings which were starting to show signs of bud expansion, were given the following treatments three days prior to being moved to a greenhouse from a 5°C storage room: (1) 5 ml of a 10% solution of Phosphon D (tributyl-2,4-dichlorobenzylphosphonium chloride, Mobil Chemicals) in 350 ml of distilled water was applied as a soil drench: (2) 0.5% solution of B-9 (N-dimethylamino succinamic acid, Plant Products Co. Ltd.) was sprayed on to wet the buds and was repeated one week after growth started in the greenhouse; and (3) distilled water was applied as a soil drench (350 ml)

and as a spray.

Leaf anthocyanin content and elongation of three terminal shoots were measured 11 weeks after treatment commenced.

C. Experiments involving the dwarf-winged burning bush

1. Environmental factors

An investigation was undertaken to determine the effects of a short photoperiod, soil moisture stress, low soil pH, and various temperatures on anthocyanin synthesis.

This research involved plants that were 45 cm tall grown in the greenhouse. Two split-plot design experiments were set up using the following treatments: (1) normal photoperiod (13 hours plus) and soil moisture maintained at 25% available water capacity; (2) normal photoperiod and soil moisture maintained at 75% available water capacity: (3) 13 hour photoperiod and soil moisture at 25% available water capacity; and (4) 13 hour photoperiod and soil moisture maintained at 75% available water capacity.

All treatments had three replicates consisting of two plants each. Treatments started on July 10, 1972. Treatments are referred to as normal photoperiod - low moisture, normal photoperiod - high moisture, short photoperiod - low moisture, and short photoperiod - high moisture, respectively, in the text.

This experiment was conducted using two temperature regimes in separate greenhouse compartments. The low temperature regime provided a day temperature of 15°C and

a night temperature of 10°C, whereas, the high temperature regime was 15°C and 18°C, respectively.

Black cloth was used for shading to provide the 13 hour photoperiod. This was reduced to 12 hours on August 10, 1972.

One additional treatment was used in the low temperature greenhouse. This consisted of normal photoperiod and the use of a growing medium of soil: peat: perlite, 2:1:2 v/v/v, with a pH of 5.2 and is referred to as low pH in the text.

Available water capacity is defined as the difference between field capacity and permanent wilting percentage (lower limit of available water).

The field capacity and permanent wilting percentage of the soil mixture was determined using the 1/3-atmosphere percentage method and 15-atmosphere percentage method, respectively, as described by the U.S. Salinity Laboratory Staff (1954).

Bouyoucos absorption blocks were calibrated for the soil mixture using the method of Bouyoucos and Mick (1940). Prior to commencement of treatments, Bouyoucos absorption blocks were placed 15 cm below the soil surface equadistant to the two plants in each plot and allowed to reach equilibrium. Electrical resistance of each block was measured twice-weekly using a Model BN-2B Bouyoucos Moisture Meter and watering was adjusted accordingly.

2. Effects of light quality

Potted one-year-old rooted cuttings and leaf discs were used to study the effect of various light spectra on anthocyanin synthesis. Three experiments were involved in this study. First, individual light cabinets were used with potted plants. Floating leaf discs were substituted for the potted plants in the second experiment. The third part of the study involved the use of a controlled environment chamber in conjunction with various broad band filters placed over potted plants.

The individual light cabinets and filters used in the first two parts of this study were primarily those designed and described by Zalik and Miller (1960) and modified by Ang (1966). Lamp wattage and the distance between lamps and plants were adjusted so that each cabinet had a spectral maximum of 10 $\mu W/cm^2/m\mu$. The light spectrum of each filter was measured at leaf level using an ISCO Model SR Spectroradiometer and is shown in Figure 1.

Individual light cabinets were used firstly with potted plants and secondly with leaf discs. Two potted plants were used in each cabinet. Leaf discs were cut as described earlier and floated in petri dishes on their adaxial surfaces in 0.3 M sucrose solution. Other growing conditions were a 12-hour photoperiod and temperatures varying between 13° and 15°C. Each light treatment was given to 3 replicates of 10 leaf discs each. Anthocyanin

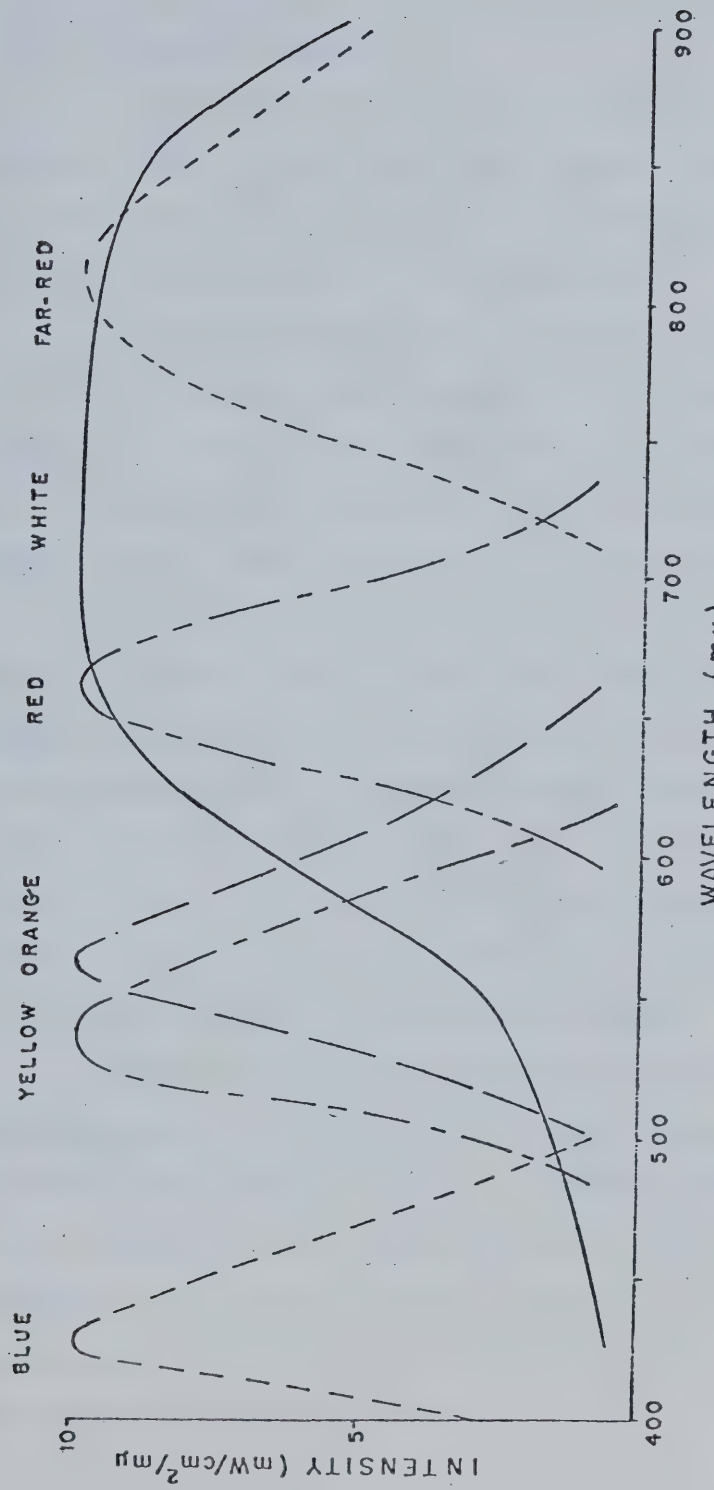


Figure 1. Spectra of light filters on individual cabinets. Intensity was measured at intervals of 25 mμ.

content of the leaves and leaf discs was measured 21 days after treatment commenced.

In the third experiment, anthocyanin content was measured after potted plants were exposed for 21 days to various light spectra of higher intensities than was available in the previous two experiments.

The potted plants were grown in a controlled environment chamber and subjected to a 12-hour photoperiod of 19,500 lux from cool-white fluorescent tubes and a day and night temperature of 15°C and 10°C, respectively. The light spectrum is shown in Figure 2.

In the continuation of this experiment broad band filters were used to break the former spectrum into segments. These filters were constructed out of 5-20 x 20 cm plexiglass pieces. They were taped together using black plastic tape to form open cubes and were inverted over single plants. Four 0.5 cm holes 1 cm below the upper edges of two opposite sides allowed air to circulate through the filters in the up-draft chamber.

Each light treatment contained two replicates. Treatments were randomized within the chamber and their positions were rotated weekly. Distances between light source and leaf level were adjusted to give the required spectral intensities as shown in Figure 2. The color designation used in the text and composition for all the filters are included in Table 1.

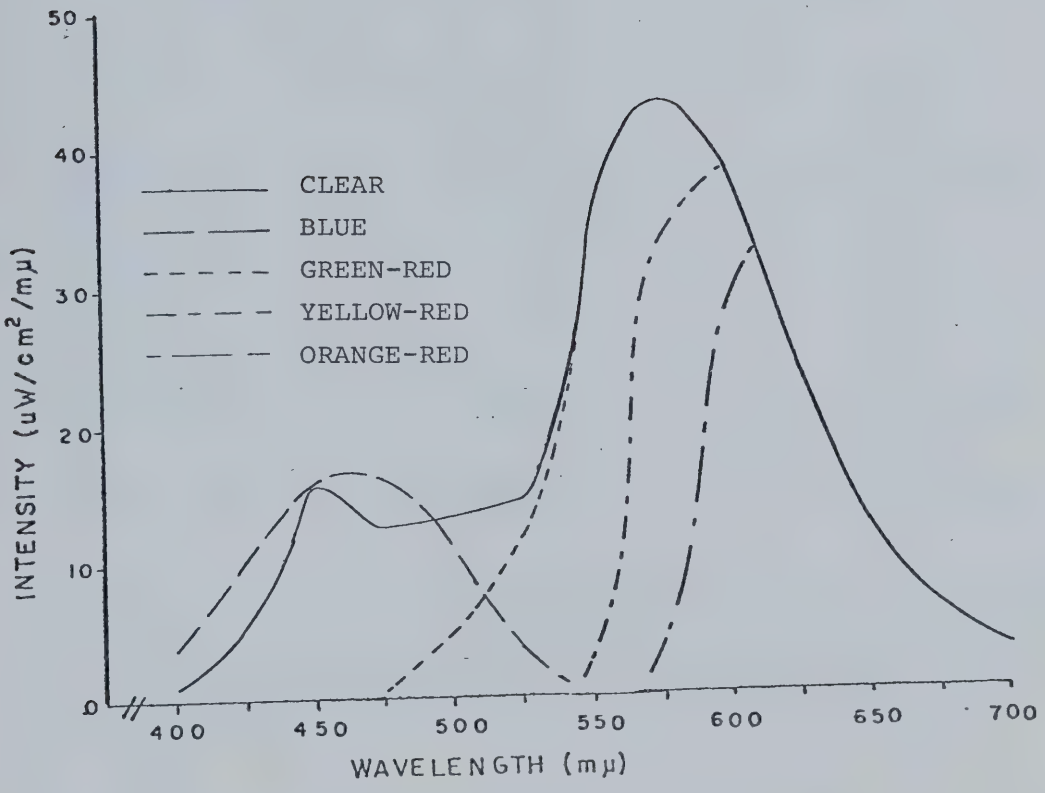


Figure 2. Spectra of cubical light filters placed over plants. Intensity was measured at intervals of 25 mμ. Color designation refers to that given in Table 5.

TABLE 1

Color designation, composition, and transmittance data for cubical light filters

| Color | Wavelengths (mμ) transmitted[a] | Plastic designation[b] |
|---|---|---|
| Clear | 400-750 | Clear |
| Blue | 400-550 | Blue 2424 |
| Green-red | 475-750 | Yellow 2208 |
| Yellow-red | 525-750 | Yellow 2086<br>Yellow 2208 |
| Orange-red | 550-750 | Red 2085<br>Yellow 2208 |

[a] - Transmittance below 400 mμ was not measured. Intensities transmitted beyond these limits was less than 2 $\mu W/cm^2/m\mu$ Transmittance was measured under cool-white fluorescent lamps.

[b] - These plastics were sold under the trade name of "Plexiglass" (Rohm and Haas Co.).

3. Rooted cuttings

Rooted cuttings were studied to determine if the original position of the cutting on the shoot affected the synthesis of anthocyanin in the rooted cutting.

Cuttings of the previous years wood were taken in May, 1972, and consisted of two types: (1) terminal cuttings, and (2) basal cuttings (that is, shoots were essentially cut into 2 pieces and identified accordingly).

Cuttings of the current season's growth were taken July 6, 1973, from a plant growing on the University of Alberta Campus. These cuttings also consisted of terminal and basal types.

All cuttings were approximately 8 cm long and were rooted in perlite under intermittent mist for nine weeks. Those cuttings taken in 1972 were observed for the presence or absence of anthocyanin pigmentation 3 weeks after potting. Cuttings taken in 1973 were analyzed for anthocyanin content of the terminal leaves and the oven dry weight of the roots was also determined. The time of analysis in 1973 corresponded to that in 1972.

## RESULTS AND DISCUSSION

### I. Experiments Involving the Schubert Chokecherry

#### A. Relative leaf position on shoot

Basal leaves were found to contain almost eleven times as much anthocyanin as terminal leaves on the same Schubert chokecherry shoot. Analysis was done at the time when terminal leaves appeared to lack anthocyanin pigmentation but basal leaves were fully colored. It must be realized that the leaves develop as the shoot elongates. Consequently, the basal leaves may be fully expanded before the terminal leaves are formed. Thus, the basal leaves were clearly more mature than the terminal leaves.

At the time of analysis, the leaves of the terminal segment, number 5, appeared to be fully expanded. Although the leaves from segment 4 and 5 did not show visible signs of anthocyanin pigmentation, they had already started to synthesize this pigment. Leaves from both of these segments contained 3.5 µg of anthocyanin per $cm^2$ (Figure 3).

A marked increase in anthocyanin content was noted in leaves from segment 3 compared to the more terminal segments. These leaves contained 9.1 µg more pigment per $cm^2$ than did leaves from segment 5. A still greater increase was noted in segment 2. The anthocyanin content

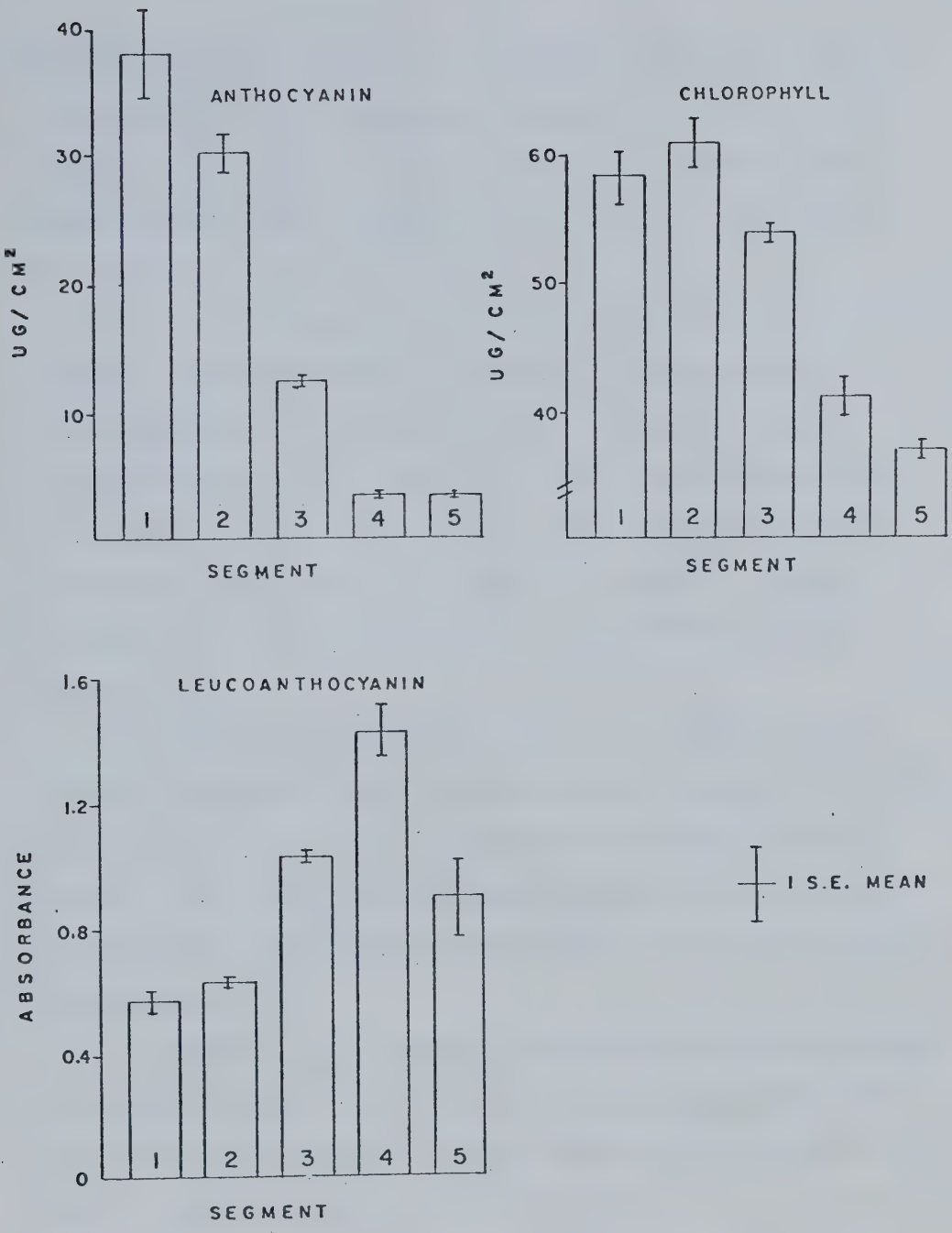


Figure 3. Anthocyanin, chlorophyll and leucoanthocyanin content of leaves from five segments of *Prunus virginiana melanocarpa* 'Schubert' shoots. Segment 1 represents the basal segment and segment 5 the terminal segment. These leaves appeared fully pigmented with anthocyanin at the time of analysis.

of these leaves increased 17.7 $\mu g/cm^2$ over that measured in segment 3. The anthocyanin content of leaves from segment 1, as shown in Figure 3, was the highest of all segments, but only increased 7.8 $\mu g/cm^2$ over that found in segment 2.

The anthocyanin content of leaves from the various segments, when compared as in Figure 3, would appear to fit a sigmoid curve. That is, the anthocyanin content increased gradually in the leaves from the terminal end to the mid section of the shoot. Then a marked increase was noted in the next one-fifth of the shoot. This was followed by a lesser increase in the remainder of the leaves.

These results indicate that the leaves of the Schubert chokecherry must reach a certain stage of maturity before anthocyanin start to accumulate rapidly in the leaves. This rapid accumulation decreased later in more mature leaves and possibly even levels off during a later stage of maturity.

Results from preliminary experiments not reported here indicated that anthocyanin is not synthesized until leaves reached a certain stage of maturity and, furthermore, does in fact decrease in mature leaves.

Total chlorophyll and leucoanthocyanin content of leaves from the various segments were also measured. The chlorophyll content, as shown in Figure 3, increased with leaf maturity in segments 5 to 2. However, a

lesser amount, that was not statistically significant, was noted in segment 1. The total chlorophyll content of the leaves showed a marked increase in concentration at a slightly earlier stage of maturity than did that of anthocyanin. It is not known if this increase in chlorophyll content was partially a result of the presence of anthocyanin or was rather the normal increase in chlorophyll content associated with leaf maturity.

Blank (1958) noted that anthocyanin-pigmented leaves contained more chlorophyll than did green types. However, with the Schubert chokecherry leaves studied, it is a case of a difference in leaf maturity and not of a difference in leaf type.

The change in leucoanthocyanin content of leaves relative to their position on the shoot was found to be essentially the opposite to that of anthocyanin and chlorophyll (Figure 3). Leucoanthocyanin content was highest in leaves from segment 4 and lowest in those from segment 1.

This data, as shown in Figure 3, indicates that the leucoanthocyanin content is relatively higher in young leaves than in the most mature leaves. However, the content of this compound increases as young leaves become more mature and then rapidly declines and likely remains fairly constant in mature leaves.

These results are consistent with those reported by Hillis and Swain (1959). They noted that leaves of

*Prunus domestica* var. Victoria increased in leucoanthocyanin content as the growing season progressed and then decreased with maturity. Similarly, the leucoanthocyanin content of the fruit skin decreased as the fruit reddened. This suggests that leucoanthocyanins may be linked with anthocyanin synthesis or at least be an indicator of the leaf's state of maturity.

B. Leaf net assimilation efficiency

From the results of the experiment previously described, it was apparent that the chlorophyll content of leaves showing distinct anthocyanin pigmentation was considerably higher than that found in younger leaves not showing such intense anthocyanin pigmentation. Therefore, the question naturally arose as to the effect of anthocyanin pigmentation on the efficiency of leaves to assimilate $CO_2$. Consequently, the photosynthetic and respiratory rates of anthocyanin and non-anthocyanin pigmented leaves were measured using a light intensity of $3.4 \times 10^5$ ergs/cm$^2$/sec. at the leaf level.

The data presented in Table 2 shows that both photosynthetic and respiratory rates were considerably higher in green leaves than in leaves containing anthocyanin. When the rates were expressed on the basis of unit area, the rates were almost 75% higher in green leaves than in reddish-green leaves. An even greater difference was noted when these rates were expressed using unit chlorophyll content as a basis. The respective

TABLE 2

Photosynthetic and respiratory rates of anthocyanin pigmented and non-pigmented *Prunus virginiana melanacarpa* 'Schubert' leaves +

| | Rates expressed as mg $CO_2/cm^2/h$ | | |
|---|---|---|---|
| | Photosynthesis | Respiration | Net Assimilation |
| Non-pigmented leaves | 0.205 | 0.037 | 0.168 |
| Anthocyanin pigmented leaves | 0.122 | 0.021 | 0.101 |
| | Rates expressed as mg $CO_2$/mg chlorophyll/h | | |
| | Photosynthesis | Respiration | Net Assimilation |
| Non-pigmented leaves | 4.80 | 0.86 | 3.94 |
| Anthocyanin pigmented leaves | 0.022 | 0.004 | 0.018 |

+ - Non-anthocyanin pigmented leaves contained 42.7 µg chlorophyll (a and b)/$cm^2$. Pigmented leaves contained 77.7 µg chlorophyll (a and b)/$cm^2$ and 17.3 µg anthocyanin/$cm^2$.

rates were then shown to be over 200 times as great in leaves not containing anthocyanin as in those that did.

It must be remembered that the pigmented leaves are older than the non-pigmented leaves. Therefore, the decreased photosynthetic and respiratory rates associated with increased anthocyanin pigmentation could be the result of increased leaf maturity rather than anthocyanin pigmentation. These results are consistent with those reported by Kozlowski (1971a) and the conclusions reached by Blank (1958). The latter researcher concluded that photosynthesis is reduced in pigmented leaves as a result of the filtering effect of anthocyanins in the epidermal cells.

The higher chlorophyll content noted in the partially anthocyanin pigmented leaves (Table 2) did not result in these leaves having an increased rate of photosynthesis compared to non-pigmented leaves. This may not have necessarily been due entirely to the anthocyanin pigmentation because the rate of photosynthesis may not be proportional to the chlorophyll content. In fact, several authors have reported that there is no correlation between the rate of photosynthesis and the chlorophyll content of leaves (Gabrielsen, 1960; Leopold, 1964).

There is, on the other hand, a great deal of evidence (Bjorkman and Holmgren, 1958; Kelskloveli and Japaridze, 1972; Shutak and Hapitan 1969) to the effect

that anthocyanin pigmentation increases the respiratory rate. This, however, was not found to be the case for the Schubert chokecherry leaves studied. The respiratory rate was found to be considerably lower for the partially pigmented leaves than for those that lacked this pigmentation.

It is not known if similar results would have been obtained if leaves with more anthocyanins had been used. However, this would necessitate the use of older leaves which are known to have a lower rate of respiration.

C. Pigment development

During the twelve-week period following the visual appearance of anthocyanins in the basal leaves of Schubert chokecherry shoots, the anthocyanin content of the fourth basal leaf increased progressively. The weekly increase of this pigment was generally double during the first three weeks, dropped off sharply in the fourth week, and resumed the original rate of increase in the fifth week. The subsequent increase to 64.6 μg per $cm^2$ (Figure 4) at the end of the period studied was very gradual.

The results noted above generally agreed with those reported earlier (Section 1A). It was observed that the anthocyanin content increased rapidly in leaves after reaching a level of approximately 5 $\mu g/cm^2$ (Figure 3). Maximum anthocyanin content was noted in the more mature leaves. However, the rate of increase of this pigment

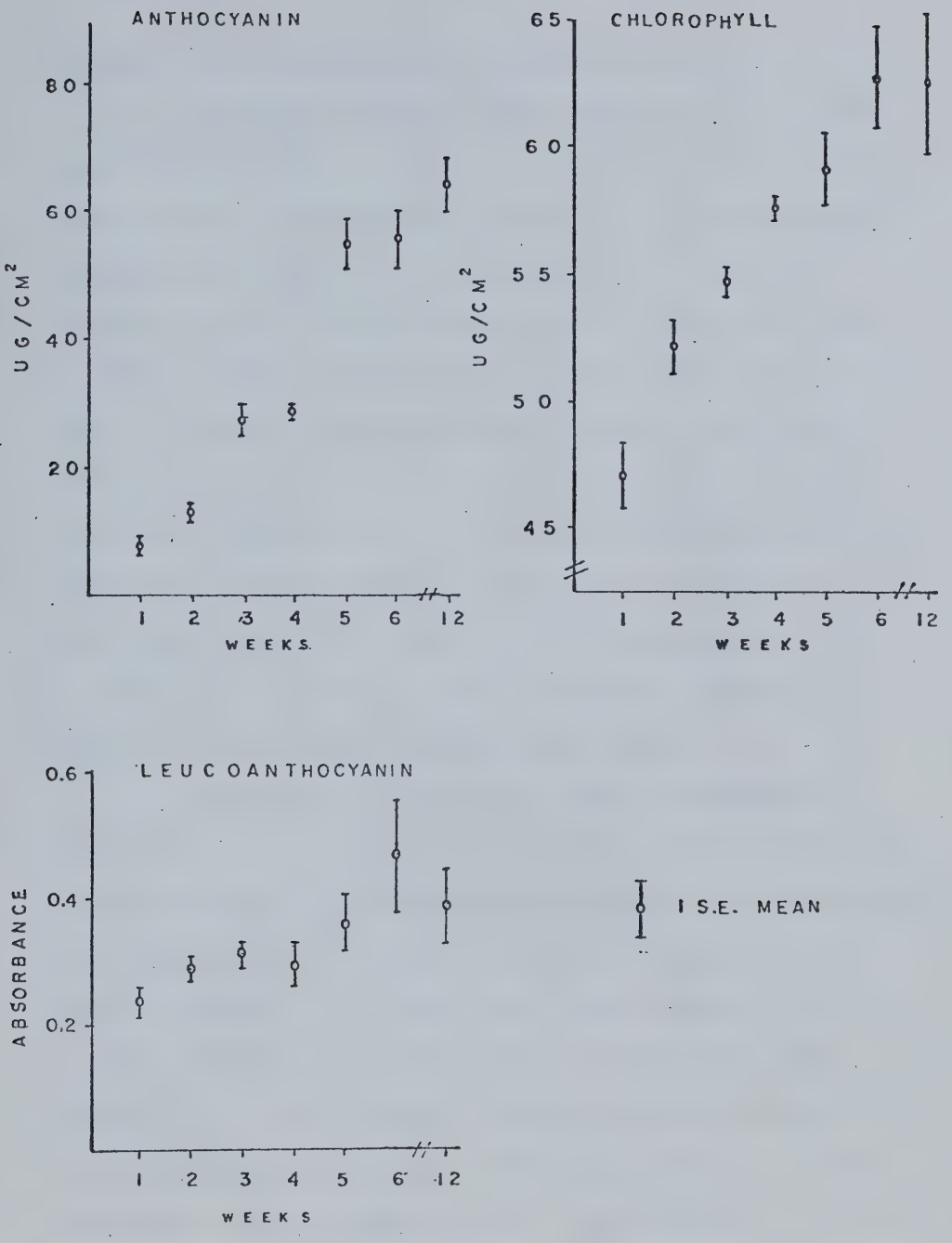


Figure 4. Pigment development in *Prunus virginiana melanocarpa* 'Schubert' leaves. Week 1 corresponds to approximately 5 1/2 weeks after the onset of bud expansion.

decreased at this stage of leaf maturity.

Although there was this similarity in results from the two studies, differences were noted. In this study, it was observed that anthocyanin content almost reached its maximum by the fifth week of the study following a very slight change during the third week. No such intermediate stage was noted during the earlier study. This can be accounted for by the fact that the stages of leaf maturity corresponding with the various leaves on a shoot cannot be directly correlated with the stages of maturity a given leaf attains at weekly intervals. This is a result of the obvious fact that the Schubert chokecherry does not grow a new leaf at weekly intervals during the growing season.

Meteorological data, as shown in Appendix 1, indicates that the fourth week, that is July 3rd to 10th had precipitation on 6 days. Furthermore, the second and third day of this week had relatively low amounts of bright sunshine. The amount of light received by leaves is known to be an important factor influencing the synthesis of anthocyanins. This, coupled with the fact that following rainy days, anthocyanin synthesis tends to be more rapid (Walter, 1967), may account for the very slight increase in anthocyanin content during the third week as well as the marked increase noted during the fourth week.

During the period studied, leaves were showing definite red pigmentation by the second week. At this stage it was noted (Figure 2) that the level of chlorophyll in the tissue was 52.2 $\mu g/cm^2$ while the anthocyanin concentration was 13.5 $\mu g/cm^2$.

The total chlorophyll content, like that of anthocyanin, increased most rapidly during the first few weeks of the test period. The maximum weekly increase of this pigment must have occurred prior to the test period and may have been at the time of the onset of the deep green color associated with the growth and development of the pallisade cells and their chloroplasts.

The chlorophyll content reached a maximum after six weeks of analysis and started to decrease by the twelfth week. The maximum total chlorophyll content measured was 62.8 $\mu g/cm^2$. It is apparent that chlorophyll degradation started prior to the anthocyanin content reaching its maximum of 64.6 $\mu g/cm^2$. Although the chlorophyll continued to mask the anthocyanins during the period studied, the masking was not as significant during the latter eight weeks of the study period.

Leucoanthocyanins, according to Robinson (1967), are precursors of anthocyanins in some plants. If this is the case, one would expect the amount of this compound to decrease as anthocyanins are synthesized. However, the results of this study, as presented in Figure 2, indicate that this is not the case for the Schubert

chokecherry. This does not rule out the possibility that leucoanthocyanins may also be synthesized during the period of anthocyanin pigmentation and this amount used for anthocyanin synthesis.

The level of leucoanthocyanin increased during the first three weeks of the period and then showed a slight decrease the fourth week. Subsequently, the level of this compound increased during the next two weeks and then showed a decrease by the twelfth week.

The decrease in leucoanthocyanin content noted during the fourth week, as shown in Figure 2, was concurrent with a decreased rate of accumulation of anthocyanins. However, it is not possible to construe from these results what the role of leucoanthocyanins is. This compound, nevertheless, may be involved in the biosynthesis of tannins, as suggested by Harborne (1964) or be an end product of another pathway (Robinson, 1967).

D. Effects of phloem disruption

Phloem disruption or ringing consisted of the removal of a strip of bark below the terminal bud on branches at the onset of bud expansion. This exposed ring of wood tissue was covered with a wound dressing to prevent moisture loss. Leaves grown above the rings on shoots which did not show phloem regrowth were analyzed for anthocyanin and chlorophyll content.

Five weeks after the branches were ringed, the

anthocyanin content of treated leaves, that is, leaves growing above the ring, was found to be higher than that of comparable leaves on ungirdled branches although the difference was not statistically significant (Table 3). However, by the sixth week after treatment, the treated leaves contained considerably more anthocyanins than did untreated leaves. Ten weeks after treatment, the treated leaves contained almost twice as much anthocyanins as did those untreated. Treated leaves, at this time, contained 100.9 μg of anthocyanins per $cm^2$ whereas untreated leaves contained only 50.9 $\mu g/cm^2$. At this stage, it was noted (Table 3) that the level of total chlorophyll in the treated leaves was 27.1 $\mu g/cm^2$. In contrast, untreated leaves contained 64.3 $\mu g/cm^2$.

Chlorophyll degradation was clearly evident in treated leaves by the end of the period. These leaves showed almost unmasked anthocyanin pigmentation ten weeks after treatment. Further chlorophyll degradation and intense red coloration was evident following the test period but was not measured. It was also noted that the treated leaves abscised about three weeks earlier than did untreated leaves.

The early coloration and maturing of the treated leaves is consistent with results attained by the use of the horticultural practice of ringing fruit trees, especially apples, to produce earlier, larger and better fruit.

TABLE 3

Anthocyanin and chlorophyll content of *Prunus virginiana melanocarpa* 'Schubert' leaves 5, 6 and 10 weeks after girdling +

| Weeks after treatment | Anthocyanin content ($\mu g/cm^2$) | |
|---|---|---|
| | Control | Girdled |
| 5 | 9.0 | 16.7 |
| 6 | 14.0[a] | 24.1[a] |
| 10 | 50.9[b] | 100.9[b] |

| Weeks after treatment | Total chlorophyll content ($\mu g/cm^2$) | |
|---|---|---|
| | Control | Girdled |
| 10 | 64.3[b] | 27.1[b] |

+ - Girdling was done at the time of bud expansion and corresponded to May 12, 1973.

- Data presented are the means of 4 replicates.

[a] - Denotes a significant difference of means from one another at the 5% level of probability as determined by a paired t-test.

[b] - Denotes a significant difference of means from one another at the 1% level of probability as determined by a paired t-test.

Many authors (e.g. Stoltz and Hess, 1966; Luckwill, 1970) have reported that ringing increases the concentration of carbohydrates and alters the level of growth regulators above the phloem disruption. The basipetal translocation of carbohydrates, as well as that of growth regulators, is impeded. These substances, according to Kozlowski (1971b), diffuse into the xylem and are translocated acropetally.

The accumulation of carbohydrates, particularly sugars, is known to be associated with increased anthocyanin synthesis. Street and Cockburn (1972) regard this increased synthesis to be the result of the greater availability of substrate material for such synthesis.

Increased anthocyanin pigmentation is also associated with high auxin levels. Walter (1967) reports that many auxin-like compounds have been used to stimulate synthesis of anthocyanins in fruit crops. Wilson (1970) reports that increased auxin concentrations have been noted above the phloem disruption. This may have been a factor contributing to the increased concentration of anthocyanins measured in the treated leaves.

Early senescence of the treated leaves was manifested by the premature degradation of chlorophyll. This phenomenon may have been the result of either moisture stress or the decreased levels of cytokinins above the ring.

Noel (1970) reports that a water stress condition may occur above the site of ringing as a result of occlusion of the xylem by tyloses. Skene (1972) concludes that this stress condition may result in the inactivation of cytokinins. Therefore, the advanced chlorophyll degradation which resulted in the low chlorophyll concentrations noted in treated leaves may have been caused by a decreased cytokinin concentration in treated shoots (Sitton *et al*, 1967).

E. Effects of root pruning

It was noted that when Schubert chokecherry trees approximately four meters tall were transplanted with a greatly reduced root system but with little or no top pruning, these trees developed red leaves very early subsequent to planting. Therefore, experiments were conducted to find out if the use of severe root pruning (i.e. transplanting) and the timing of it did have an effect on the amount of anthocyanins synthesized. Trees were given the following three treatments: (1) severe root pruning the previous fall; (2) severe root pruning in the spring; and (3) control. Leaves were analyzed when the control leaves showed slight pigmentation.

The results in Table 4 show that leaves from trees root - pruned in the spring had more than triple the concentration of anthocyanins than did leaves from the control trees. Leaves from trees treated the previous fall contained more anthocyanins per leaf area than did

TABLE 4

Influence of severe root pruning on anthocyanin content and size of leaves from *Prunus virginiana melanocarpa* 'Schubert' trees+

| | Anthocyanin $\mu g/cm^2$ | Leaf Area $cm^2$ |
|---|---|---|
| Established Trees | 13.54[a] | 26.27[a] |
| Fall Transplants | 20.86[b] | 17.62[b] |
| Spring Transplants | 54.09[c] | 10.58[c] |

+ - Severe root pruning consisted of uniform pruning and transplanting of trees with little or no top pruning.

[a] - Data presented are means of 4 replicates. Means followed by the same letter are not significantly different from one another at the 5% level of probability as determined by Duncan's new multiple range test.

the control trees.

Leaves from trees treated in the spring had synthesized almost their maximum amount of anthocyanins (Figure 3 and Table 3) by the time the control trees were just starting to show red coloration. The spring transplanted trees, with their greatly reduced root system were at a disadvantage in the uptake of soil water and mineral nutrients (Wareing, 1970). The production of smaller leaves (Figure 5) in conjunction with the occurrance of earlier pigmentation of spring treated trees is consistent with reports that root growth and the availability of soil moisture may influence both leaf growth and leaf metabolism (Blank, 1958; Kongsrud, 1969; Stanhill, 1957; Toy, 1969).

Blank (1958) states that drought may enhance autumn leaf coloration of some plants but not of all plants studied. This is in agreement with Toy's (1969) report that *Acer truncatum* trees grown in a dry area of California developed better autumn coloration when summer irrigated than did non-irrigated trees. Toy found that irrigation delayed autumn coloration by four to five days but had no effect on the average color of the leaves.

Therefore, it seems quite possible that the increased anthocyanin content detected in leaves of trees subjected to severe root pruning was the result of earlier

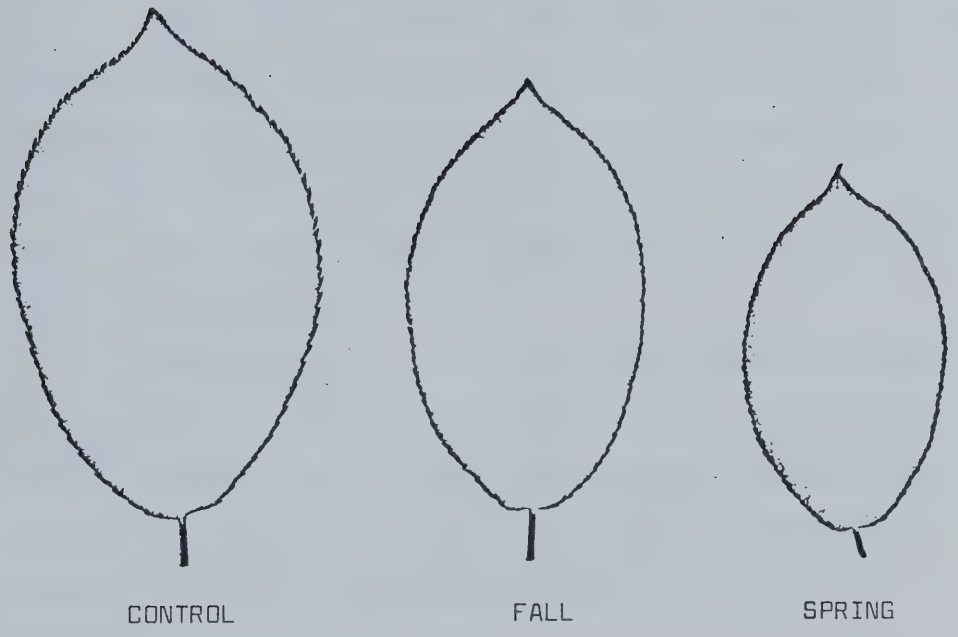


Figure 5. Effects of severe root pruning and season of root pruning on leaf size of *Prunus virginiana melanocarpa* 'Schubert'.

as well as accelerated synthesis of anthocyanins in these leaves. The final peak coloration, however, may not have been more intense than that attained by the control trees at a later date.

The greatly reduced root system and the resulting moisture stress of spring treated trees may have been associated with restricted synthesis and translocation of cytokinins and some gibberellins (Cleland, 1969; Wareing, 1970). This could influence shoot growth as well as leaf growth and metabolism.

Wareing (1970) notes that shoot and root growth must be kept in balance since the roots are responsible for the factors such as cytokinins and some gibberellins. Similarly, Kozlowski (1971a) states that a water deficit can result in decreased shoot elongation and leaf expansion.

F. Effects of anti-gibberellin-like growth regulators

Gibberellins have been associated with stem elongation in many plants (e.g. Marth *et al*, 1956; McVey and Wittwer, 1958). These compounds have also been known to delay autumn coloration (Brian *et al*, 1959). Growth retardants which act as anti-gibberellins, that is ones whose effects can be reversed by the application of gibberellins, are a useful tool in studying the biosynthesis and physiology of gibberellins (Lang, 1970).

The results, as shown in Table 5, indicate that neither phosphon nor B-9, two anti-gibberellin-like

TABLE 5

Effect of anti-gibberellin-like growth regulators on shoot elongation and on synthesis of anthocyanin by *Prunus virginiana melanocarpa* 'Schubert' +

| Treatment | Anthocyanin Content ($\mu g/cm^2$) | Elongation (cm) |
|---|---|---|
| Phosphon | 67.7[a] | 8.5[a] |
| B-9 | 58.2[a] | 11.3[a] |
| Control | 51.7[a] | 16.9[a] |

+ - Data represents the means of 3 replicates measured 11 weeks after treatment commenced.

[a] - Means followed by the same letter are not significantly different from one another at the 5% level of probability as determined by Duncan's new multiple range test.

compounds (Pharis *et al*, 1970; West and Fall, 1970), appreciably increased the leaf content of anthocyanins or decreased shoot elongation.

The lack of statistically significant differences between treatments probably was the result of using saplings that were top pruned when potted at the time of the onset of dormancy the previous fall. As a result of this prior treatment, the terminal buds were not morphologically uniform.

Even though the results are not statistically different, the data does indicate that the anti-gibberellin-like compounds used did tend to increase the anthocyanin content, and to decrease the shoot growth. It also was observed that the number of leaves was not reduced on the treated plants. Consequently, the internodes were shorter on treated plants than on those on the control plants.

Earlier experiments revealed that leaf coloration was delayed until the leaves reached a certain stage of maturity or were no longer on shoot sections near the site of active stem elongation. From the effects of two anti-giberellin-like compounds it was not apparent that the delay in leaf coloration was a possible result of a higher gibberellin content in these shoot sections.

## II. Experiments Involving the Dwarf-winged Burning Bush

### A. Environmental factors

The first investigation of environmental factors influencing anthocyanin synthesis included the effect of

photoperiod, soil moisture stress, soil pH, and temperature.

Leaves on plants grown in a greenhouse under either normal light conditions for this area or under a 12 hour photoperiod did not show red coloration prior to abscission. Similarly, leaves on plants grown under soil moisture stress conditions or with adequate soil moisture did not show red coloration. Soil pH of 5.8 or 6.5, likewise, did not influence leaf coloration. Neither of the temperature regimes used in conjunction with the other growing conditions used, stimulated anthocyanin synthesis.

Even though soil moisture stress and neither of the temperature regimes had any visible effects on red coloration, they did affect the growth of the leaves. Leaves on plants grown under a soil moisture stress condition developed lighter green pigmentation than did those receiving adequate moisture. Leaves on plants grown under the high temperature regime abscised over five months later than did leaves on plants grown under the low temperature regime.

Moisture stress conditions are known to influence nutrient uptake and metabolism. Kongsrud (1969) reports that the growth of leaves and their metabolism may be influenced by the availability of soil moisture. Cleland (1969) associated the influence of the availability of soil moisture with the movement of mineral nutrients. Therefore, it seems apparent that the moisture stress experienced by plants given the low moisture treatment was responsible for

a decreased uptake of nutrients. Nitrogen uptake may then have been slightly deficient and resulted in light green leaves on these plants.

The delayed leaf senescence and abcission observed on plants grown under the high temperature treatment is consistent with results published by Abbot (1970) and Tromp (1970). These authors noted that leaf senescence and abscission is usually induced by low temperatures. Thus, it is logical that leaf senescence would be delayed by high temperatures.

From the results of the experiment, it was apparent that the synthesis of anthocyanins could not be induced in leaves of plants grown in a greenhouse and exposed to the natural summer and fall photoperiods, or to a 12-hour photoperiod, nor both photoperiods in conjunction with the high and low temperatures. Also, neither high nor low moisture levels in conjunction with these treatments induced anthocyanin synthesis. However, it was not known from these results if lower growing temperatures or different light conditions would have induced anthocyanin synthesis. Therefore, experiments were carried out to elucidate the latter.

B. Effects of light quality

The effects of various light spectra on anthocyanin synthesis proved to be most interesting.

Potted plants grown under white light and those

grown under narrow light spectra failed to develop anthocyanin pigmentation for the six week duration of the experiment. However, the latter group of plants abscised their leaves during the experiment.

All leaves that abscised became chlorotic prior to abscission except those receiving blue light. Plants grown under the yellow, orange, and far-red light filters received especially low light intensities (Figure 4) in those portions of the spectrum (400 to 500 mμ and 600 to 700 mμ) where both chlorophyll synthesis and photosynthesis are known to occur (Blackwell, 1966). The light intensity reaching the plants through the red filter may also have been too low for either of these plant responses to have occurred. Leaves of plants grown in blue light may have synthesized chlorophyll but possibly did not receive enough light intensity for photosynthesis to occur and, therefore, also abscised their leaves.

In order to overcome the problem of leaf abscission, a further study was undertaken using leaf discs floated on their adaxial surfaces in a 0.3 M sucrose solution in petri dishes. These leaf discs, unlike intact plants, did synthesize anthocyanins under certain light spectra.

Data presented in Table 6 indicates that leaf discs exposed to white light, synthesized appreciably more anthocyanins than did those exposed to blue light. The anthocyanin contents resulting from these two treatments

TABLE 6

Anthocyanin content of *Euonymus alatus compactus* leaf discs floated on a 0.3 M sucrose solution and exposed to various light spectra+

| Light Spectra | Anthocyanin Content ($\mu g/cm^2$) |
|---|---|
| White | 6.6 |
| Blue | 3.6 |
| Yellow | 2.3 |
| Orange | 1.1 |
| Red | - |
| Far-red | - |

+ - Leaf discs were floated on their adaxial surfaces and exposed to the various spectra shown in Figure 1 for 21 days.

were 6.6 and 3.6 $\mu g/cm^2$, respectively. A statistically significant decrease in anthocyanin content was noted when leaf discs were exposed to light spectra of longer wavelengths. Leaf discs exposed to red or far-red light failed to synthesize anthocyanins.

These results tend to agree with results reported by Creasy (1968) which showed that strawberry leaf discs floated on a sucrose solution had a higher phenylalanine ammonia-lyase (PAL) activity than did those floated on distilled water. Grisebach (1965) reported that PAL is a major enzyme component of the pathway by which anthocyanins are synthesized.

It may be noted that the leaf discs exposed to white light received more than twice as much total light energy as did those exposed to any other light source (Figure 1). In addition to this, the white light was low in orange light and contained even less yellow light and next to no blue light. Therefore, the increased synthesis of anthocyanin noted in leaf discs exposed to white light compared to those exposed to other light sources could be attributed to an exposure to higher light energy. However, this was not the case for the difference in anthocyanin content noted in leaves exposed to the various other light spectra. These results may indicate part of the action spectrum for anthocyanin formation in leaves of the dwarf-winged burning bush.

Certainly, they do indicate that light quality and light intensity are important factors in anthocyanin synthesis.

The synthesis of anthocyanin by leaf discs floated in sucrose solution is consistent with other research on this plant reported by Lee and Tukey (1971). These researchers observed that feeding sucrose solution to leaf discs obtained from either normal leaves or leaves from which sucrose had been leached, significantly increased anthocyanin synthesis.

The availability of sucrose, and possibly of other metabolites, may have been a limiting factor in the synthesis of anthocyanins in leaves of plants grown under the various light sources. However, the stimulatory effects of light of short wavelengths on the synthesis of anthocyanins was evident when leaf discs were floated on a sucrose solution. It was, therefore, decided to continue the investigation of the effects of various light spectra on synthesis of anthocyanins on intact plants using higher light intensities.

Potted plants were moved from the greenhouse to a controlled environment chamber and exposed to light from cool-white fluorescent tubes. One week later, plants started to show anthocyanin pigmentation. Two weeks after treatment commenced, pigmentation intensified but was masked by chlorophyll. The masking effect of chlorophyll was not noticeably reduced prior to leaf abscission and as

a result of this, the leaves did not develop the typical brilliant red coloration for which these plants are noted.

Even though chlorophyll degradation was not achieved in leaves grown under this light spectrum, the investigation was continued to ascertain the effects of various regions of this spectrum on anthocyanin pigmentation. Broad band filters were designed so that either the whole spectrum was included or parts of the spectrum were reduced or eliminated (Figure 2). The normal reduction in light intensity caused by the various filters was compensated for by altering the distance from the light source.

If part of the light spectrum emitted by cool-white fluorescent tubes was eliminated by placing broad band filters over the plants, visible anthocyanin synthesis did not occur (Table 7). The reduced anthocyanin synthesis in leaves grown under the clear plastic filter was an interesting development. This filter did not reduce or eliminate any part of the test spectrum. It is possible that short wavelengths, that is those less than 400 mμ and not able to be measured with the instrument used, were either reduced in intensity or filtered out completely by the clear plastic.

Collingbourne (1966) reports that the shortwave ultra-violet portion of sunlight, that is wavelengths shorter than 290 mμ, does not reach the earth's surface. It is, therefore, not a factor in the synthesis of anthocyanins in plants grown under normal conditions.

TABLE 7

Anthocyanin content of *Euonymus alatus compactus* leaves grown under various light filters+

| Filter | Anthocyanin content ($\mu g/cm^2$) |
|---|---|
| Control | 42.5 |
| Clear | 11.4 |
| Blue | - |
| Green-red | - |
| Yellow-red | - |
| Orange-red | - |
| Glass | 6.7 |

+ - Filter composition and spectra are given in Table 1 and Figure 2 respectively.

- The light source was cool-white fluorescent tubes.

- Plants were grown under the filters for 21 days.

However, it is known to have an effect on plants grown under artificial conditions. Hadwiger and Schwachau (1971) observed that this shortwave ultra-violet light stimulated the synthesis of PAL in pea tissue. Arthur (1936), in contrast, reported that shortwave ultraviolet light was deleterious to apple skin. Interestingly, he also reported that the UV portion of sunlight reaching the earth's surface and blue-violet light were very important for the development of anthocyanins in apple skin.

It is, therefore, very possible that wavelengths shorter than 400 mμ stimulate the synthesis of anthocyanins in leaves of the dwarf-winged burning bush. Furthermore, this portion of the spectrum may have been reduced by the clear plastic, thereby reducing the amount of coloration of leaves grown under it.

Ordinary window glass, such as that used in greenhouses, is known to filter out visible blue-violet and the ultra-violet of sunlight, that is, wavelengths of 313 to 290 mμ (Arthur, 1936). Assuming that light from this part of the spectrum is important for the synthesis of anthocyanin in leaves of the dwarf-winged burning bush, then plants exposed to light from cool-white fluorescent tubes filtered through one pane of window glass would be expected to synthesize less anthocyanin than plants exposed to similar but unfiltered light. This definitely proved to be true.

Leaves on plants exposed to glass-filtered light contained 6.7 μg anthocyanin per $cm^2$, whereas similar leaves exposed to non-filtered light contained 42.5 $\mu g/cm^2$ or more than six times as much anthocyanin. These results concur with the results of previous studies (Popp and Brown, 1936) and suggest that the filtering effect of window glass is, at least in part, responsible for the lack of anthocyanin pigmentation noted in leaves of plants grown in a greenhouse and given the various treatments described earlier. Furthermore, these results reveal that visible blue-violet and longwave ultra-violet light may be important for the development of anthocyanin pigmentation in leaves of the dwarf-winged burning bush.

If the light spectrum, and especially that near the lower limits of the light reaching the earth's surface, does indeed decrease with an increase in air mass (Collingbourne, 1966) as associated with changes in altitude (Caldwell, 1968), latitude, and season (Brooks and Miller, 1963; Robertson, 1966), then it is conceivable that this could be a factor in the reduced fall coloration of the dwarf-winged burning bush in the Edmonton area. Furthermore, it may partly account for the decreased synthesis of anthocyanin in leaves during rainy weather as reported by Lee & Tukey (1971).

C. Rooted cuttings

An interesting phenomenon was observed in young greenhouse grown rooted cuttings of the dwarf-winged burning

bush. Prior to these rooted cuttings going dormant their first season after rooting, it was noted that terminal cuttings developed intense red pigmentation unmasked by chlorophyll, whereas basal cuttings lacked red pigmentation. Consequently, additional cuttings were taken, rooted, and grown in a greenhouse. The phenomenon was repeated and this time the anthocyanin pigmentation was measured.

Leaves from terminal cuttings in this experiment contained 26.0 μg of anthocyanin per $cm^2$, but leaves from basal cuttings contained only 6.2 μg of this pigment per $cm^2$ (Table 8). These results indicate that the anthocyanin content of the terminal set of leaves from these two types of cuttings was generally dependent upon the relative location of the cutting on the intact parent shoot.

It was surprising to note that rooted terminal cuttings developed anthocyanin pigmentation when removed from under the mist of the propagation bed and placed in a greenhouse, whereas one-year-old and three-year-old plants growing in the same greenhouse failed, as noted earlier, to develop this pigmentation.

Lee and Tukey (1971) reported similar results, that is, misted plants of this species developed anthocyanin pigmentation when removed from under intermittent mist. Furthermore, cuttings from these misted plants rooted earlier and developed more roots than did cuttings from non-misted plants.

TABLE 8

Anthocyanin content and root growth
of rooted terminal and basal cuttings
of *Euonymus alatus compactus* +

| Type of Cutting | Anthocyanin content ($\mu g/cm^2$) | Root dry weight (mg) |
|---|---|---|
| Terminal | 26.0 | 229 |
| Basal | 6.2 | 143 |

+ - Analyses were done 3 weeks after cuttings were potted.

The question naturally arose as to the difference there might be in the weights of roots from the two types of rooted cuttings. Three weeks after the time of potting, the oven dry weights of the roots were determined. The results as shown in Table 8 indicate that the weight of roots from terminal cuttings was significantly higher than that of the roots from basal cuttings. It is not known if a more pronounced difference in root weights would have been obtained if the roots had been weighed at a different time.

The cause of the enhanced red coloration of leaves on terminal shoot cuttings over that of leaves on basal cuttings is also open to speculation. It is possible that the levels of growth hormones and other metabolites were different in the two types of cuttings. Rooting hormones were not used in this experiment and, therefore, did not contribute to the phenomenon. Metabolites, including growth hormones, are produced in leaves as well as in roots. Terminal cuttings had greater root growth and leaf area (Figure 6) than did basal cuttings. Therefore, the concentration of growth hormones and other metabolites could have been higher in terminal cuttings than in basal cuttings. This, in turn, could have been a causitive factor in the difference of anthocyanin content noted in the two types of cuttings.

Figure 6. Terminal (left) and basal (right) rooted cuttings of *Euonymus alatus compactus*. This picture was taken 3 weeks after the cuttings were potted and moved to a greenhouse.

## SUMMARY AND CONCLUSIONS

This investigation has shown that both anthocyanin and chlorophyll content of Schubert chokecherry leaves are dependent upon the maturity of the leaves. Furthermore, anthocyanin pigmentation was correlated with a low assimilation of $CO_2$ in leaves. The cultural practices employed enhanced the coloration of Schubert chokecherry leaves. However, leaves of the dwarf-winged burning bush did not color up in response to the cultural techniques used on this plant. Subsequent research with various light spectra revealed the probable reason for the failure of the greenhouse experiments with this plant.

In research on the Schubert chokecherry, it was determined that its leaves rapidly synthesize anthocyanins once the process starts. During the first three weeks after the onset of pigmentation, the anthocyanin content of leaves generally doubled each week. This was followed by a slower rate of accumulation in the fourth week and then rapid synthesis the following week. At the end of the twelfth week, these leaves contained 64.6 µg anthocyanin per $cm^2$. Chlorophyll degradation had started somewhat prior to this time since the chlorophyll content at the end of 12 weeks was slightly lower than the 62.8 µg per $cm^2$ noted during the sixth week.

A similar progression of increased anthocyanin and chlorophyll content was noted in leaves on the same shoot. Basal leaves contained almost 11 times as much anthocyanin and 60 per cent more chlorophyll than did terminal leaves. Chlorophyll degradation probably accounted for the basal leaves containing less chlorophyll than did those on the second basal segment of the same shoot.

It was not known if the chlorophyll content increased substantially to compensate for the accumulation of anthocyanins. However, measurement of photosynthetic rates and respiratory rates indicated that green leaves photosynthesized and respired 75 per cent more $CO_2$ per $cm^2$ per hr or 200 times as much $CO_2$ per mg chlorophyll per hr than did pigmented leaves which contained 17.3 µg anthocyanin per $cm^2$. Therefore, even though the pigmented leaves contained more than 80 per cent more chlorophyll than did the green leaves, the photosynthetic and respiratory rates were greatly reduced. This could be a result of the anthocyanin pigmentation, but it could also be due to the greater maturity of pigmented leaves.

The leucoanthocyanin content of leaves displayed great variability over the 12 week period studied. However, analysis of leaves from the same shoot revealed that terminal leaves, that is, the youngest leaves, had a low content of this compound. An increasing gradient of

leucoanthocyanin was observed in leaves from terminal to basal locations on the shoot with the exception of the most basal set of leaves. These contained the least amount of the compound. This gradient of concentration, when compared with an almost opposite gradient of anthocyanin concentration, suggests that this compound may be involved in the synthesis of anthocyanin. Certainly, the amount of leucoanthocyanin is an indication of leaf maturity.

Cultural practices employed greatly increased anthocyanin content in Schubert chokecherry leaves but others failed to do so in leaves of the dwarf-winged burning bush. Severe root pruning in the spring, girdling the previous year's growth, and the application of anti-gibberellin-like compounds enhanced anthocyanin pigmentation of Schubert chokecherry leaves. Furthermore, severe root pruning greatly reduced leaf growth on this plant, while girdling hastened leaf maturity and senescence.

It was not known if the development of anthocyanin pigmentation of the dwarf-winged burning bush was a photoperiodic response or not. However, manipulation of the photoperiod in combination with different soil moisture levels and growing temperatures failed to initiate the synthesis of anthocyanin.

Interestingly, however, some of the cultural practices used did affect leaf senescence and growth. Leaf abscission was delayed five months on plants grown

in the high temperature regime, while leaves on plants subjected to low moisture conditions were light green in color. The former was assumed to be the result of the lack of low temperatures which are known to stimulate senescence. A possible decreased nutrient uptake by plants subjected to moisture stress conditions may have resulted in nitrogen deficiency symptoms in the latter.

The failure of the greenhouse experiments to give positive results with the dwarf-winged burning bush led to the study of the effects of light quality on anthocyanin synthesis in leaves of this plant. Leaf discs floated on their adaxial surfaces in 0.3 M sucrose solution and exposed to various low intensity light spectra gave the first indication of what light wavelengths are important in anthocyanin synthesis in leaves of this plant. Blue light resulted in 3.6 μg anthocyanin per $cm^2$ of leaf discs. However, the concentration of this pigment decreased with longer wavelengths and was not detected in leaf discs exposed to red or far-red light. Plants exposed to these spectra failed to develop pigmented leaves and soon abscised their leaves. It seemed logical that higher light intensities would have to be used in further studies.

Preliminary experiments revealed that mature greenhouse-grown plants exposed to light emitted by cool-white fluorescent tubes started to color up one week after treatment commenced. This was a very enlightening

observation since the growing conditions were kept similar to those in the cool greenhouse. Consequently, it was concluded that part of the spectrum emitted by cool-white fluorescent tubes must be required for the synthesis of anthocyanins in leaves of the dwarf-winged burning bush. Furthermore, part of this spectrum must be reduced or eliminated from sunlight as it passes through window glass. Therefore, filters were designed to eliminate various part of the spectrum above 400 mμ. Intensities of shorter wavelengths were not measurable by the instrument used.

Anthocyanin synthesis was completely impeded by these filters. Furthermore, plants grown under a clear plastic filter had greatly reduced anthocyanin content even though this filter did not eliminate any of the measurable test spectrum. It was therefore suspected that short wavelengths (less than 400 mμ) are either eliminated or reduced in intensity by the clear plastic.

The fact that window glass is known to filter out visible blue-violet and ultra-violet of sunlight, that is, wavelengths of 313 to 290 mμ, led to the use of it as an additional filter. This was also in anticipation that it would elucidate the problem of the lack of pigmentation in the greenhouse experiment. Interestingly, leaves of plants grown under this filter contained less than one-sixth the amount of anthocyanin of the controls. Therefore, it must be concluded that light filtered out by window glass is important in the synthesis of antho-

cyanin in leaves of the dwarf-winged burning bush. Furthermore, this could account for the lack of results in the greenhouse experiment.

Propegation of the dwarf-winged burning bush by cuttings revealed that leaves on rooted terminal cuttings contained more anthocyanin than did those on rooted basal cuttings. Furthermore at the time of analysis, that is 3 weeks after potting, the oven dry weight of roots from terminal cuttings was significantly higher than that of roots from basal cuttings.

Growth regulators are known to accumulate above the site of phloem disruption and are also known to enhance root initiation. Therefore, it is possible that the difference in anthocyanin accumulation and root growth is a result of a difference in concentration of growth regulators in the two types of cuttings. This phenomenon as well as the fact that these rooted cuttings developed full anthocyanin coloration in a glass greenhouse whereas mature plants grown under similar conditions failed to synthesize anthocyanins, clearly deserves further investigation.

The lack of full autumn coloration of the dwarf-winged burning bush in the Edmonton area may be attributed to the fact that short wavelengths of sunlight are reduced in intensity by the increased air mass through which the light must pass. This is a result of the decrease in solar elevation during the fall months and would be comparible to some of the data reported by Caldwell (1968). Caldwell

found that high altitudes permitted the reception of more energy from ultraviolet light than at lower altitudes which were buffered by a larger air mass. The exact seasonal fluxes in the solar spectrum for this area are not known at this time. However, this factor in connection with the severe frosts typical in the fall may account for the lack of pigmentation prior to leaf abscission.

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APPENDIX

Table i. Meteorological Data+

| Date | Daily Temperature[a] Max. | Min. | Daily Precipitation[b] | Daily Bright Sunshine[a] |
|---|---|---|---|---|
| June 1 | 60 | 40 | | 102 |
| 2 | 66 | 36 | | 145 |
| 3 | 64 | 43 | | 141 |
| 4 | 68 | 40 | | 134 |
| 5 | 70 | 38 | T | 121 |
| 6 | 71 | 45 | T | 61 |
| 7 | 71 | 51 | | 129 |
| 8 | 71 | 39 | 01 | 95 |
| 9 | 65 | 36 | T | 144 |
| 10 | 58 | 36 | 02 | 59 |
| 11 | 63 | 41 | 01 | 109 |
| 12 | 75 | 40 | | 104 |
| 13 | 74 | 55 | T | 112 |
| 14 | 59 | 50 | 47 | 08 |
| 15 | 54 | 47 | 188 | 00 |
| 16 | 59 | 47 | 65 | 12 |
| 17 | 69 | 41 | 18 | 96 |
| 18 | 70 | 45 | 01 | 113 |
| 19 | 73 | 45 | 05 | 128 |
| 20 | 73 | 48 | 06 | 162 |
| 21 | 78 | 48 | | 124 |
| 22 | 88 | 49 | | 136 |
| 23 | 84 | 56 | | 110 |
| 24 | 69 | 52 | 137 | 00 |
| 25 | 65 | 51 | 66 | 52 |
| 26 | 71 | 47 | | 158 |
| 27 | 76 | 49 | | 145 |
| 28 | 70 | 51 | T | 32 |
| 29 | 69 | 45 | 23 | 124 |
| 30 | 58 | 47 | 21 | 05 |

(Continued)

Table i. Meteorological Data[+] (Continued)

| Date | Daily Temperature[a] Max. | Min. | Daily Precipitation[b] | Daily Bright Sunshine[a] |
|---|---|---|---|---|
| July 1 | 60 | 46 | 100 | 29 |
| 2 | 72 | 44 | | 156 |
| 3 | 74 | 48 | | 159 |
| 4 | 74 | 55 | 12 | 84 |
| 5 | 67 | 47 | 14 | 92 |
| 6 | 67 | 45 | T | 117 |
| 7 | 68 | 44 | T | 147 |
| 8 | 72 | 43 | 08 | 111 |
| 9 | 74 | 41 | 35 | 134 |
| 10 | 81 | 48 | T | 128 |
| 11 | 71 | 49 | T | 131 |
| 12 | 70 | 50 | T | 148 |
| 13 | 71 | 44 | | 144 |
| 14 | 80 | 46 | | 134 |
| 15 | 70 | 50 | T | 68 |
| 16 | 70 | 45 | T | 144 |
| 17 | 66 | 50 | 12 | 34 |
| 18 | 72 | 42 | T | 98 |
| 19 | 76 | 5 | | 149 |
| 20 | 88 | 52 | | 135 |
| 21 | 67 | 53 | 08 | 32 |
| 22 | 63 | 48 | 17 | 26 |
| 23 | 74 | 43 | 50 | 125 |
| 24 | 69 | 44 | 03 | 131 |
| 25 | 73 | 49 | | 69 |
| 26 | 74 | 55 | | 85 |
| 27 | 64 | 50 | 86 | 40 |
| 28 | 70 | 48 | | 143 |
| 29 | 77 | 47 | T | 142 |
| 30 | 80 | 55 | 01 | 145 |
| 31 | 83 | 51 | | 146 |

(Continued)

Table i. Meteorological Data[+] (Continued)

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T - Meteorological Data for the Edmonton International Airport. Canada Atmospheric Environmental Service Monthly Record. Meteorological Observations in Canada. June 1973, July 1973.

a - The unit of temperature is °F.

b - The unit of precipitation is 0.01 inches.

c - The unit of bright sunshine is 0.1 hour. These figures are based on observations made with a Cambell-Stokes Sunshine Recorder which gives a record of sunshine intense enough to scorch or burn a standard card upon which the rays of the sun have been concentrated by a glass sphere.

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